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The role of prostaglandins and nitric oxide in the response of bone to mechanical forces

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Summary

We have developed an experimental model whereby bone is exposed to a brief episode of mechanical stimulation, which is followed by bone formation. The earliest response is in osteocytes, which express c-fos and insulin-like growth factor (IGF-1) within 30–60 min. Thirty-six to 72 h after loading bone matrix gene expression occurs on bone surfaces. The osteogenic response can be suppressed by a single dose of nitric oxide synthase (NOS) or prostaglandin (PG) synthase inhibitors, if these are administered just before mechanical stimulation: similar doses after stimulation have no effect. There is a later phase of indomethacin-sensitivity associated with COX-2 expression in bone at 6 h. Thus, mechanically induced osteogenesis involves early expression of c-fos and IGF-1 by osteocytes, which are believed to be the strain-sensitive cells in bone. Both NOS and PG synthase, either in parallel or in sequence, are crucial to the initial transduction of the mechanical stimulus into an osteogenic response.

Key words: NO, Bone, Osteoarthritis.

Introduction

The primary function for which bone has evolved is to act as mechanical support. Thus, the shape of bones is determined by the genetic program, upon which is superimposed adaptation to the mechanical environment. The distinct contribution of these two influences is most clearly seen in limb bones, in which mechanical usage causes a substantial change in shape, and an increase in the quantity of bone, compared to that determined genetically.[1] Such mechanically adapted bones show a remarkably consistent strain response to mechanical usage: peak strains of 2000–3000 microstrain (μl) are observed over the cortical surface of bones in a wide variety of species during physiological activity (see [2]).

Understanding the mechanisms by which mechanical forces regulate the structure and quantity of bone could provide opportunities to mimic or amplify the responses of bone to mechanical stimuli, as a strategy to prevent fractures in diseases such as osteoporosis.

The experimental model we have developed to analyze these mechanisms exerts a compressive load on the 8th caudal vertebra (C8). A single, 10 min application of external loading, sufficient to cause strains within the physiological range, is followed by a substantial osteogenic response over the ensuing 7–10 days.[3] We have used this model to analyze the sequence of events after a temporally-defined stimulus, that leads to the initiation of bone formation.

Methods

To enable mechanical stimulation of the 8th caudal vertebra, stainless steel pins were inserted into the mid diaphysis of the 7th and 9th caudal vertebrae (C7 and C9). C8 is then loaded in compression by a cam-operated device using a load of 150 Newtons for 300 cycles at 1 Hz. This regimen was found, using rosette strain gauges, to induce a peak strain of 700 μl, over the cortex of the loaded vertebra.[4] As controls, C8 from untreated rats, and C8 from animals that were pinned but not loaded, were used. Additional internal controls comprised C6, which is equidistant with C8 from the loading pin, but not exposed to mechanical stimulation by the loading device.

For assessment of bone formation rates, rats were injected with fluorochrome labels at intervals after mechanical stimulation. These measurements of bone formation dynamics were complemented by static measurements, such as the proportion of bone surfaces covered with osteoblasts and osteoclasts. Where appropriate, vertebrae were prepared for Northern analysis of extracted RNA, or for *in-situ* hybridization. For *in-situ* hybridization animals were perfusion-fixed at intervals after mechanical stimulation. Vertebrae were then embedded in paraffin, sectioned, and subjected to standard *in-situ* hybridization protocols, using cDNA or riboprobes.

Results and discussion

We found that a single, 5-min episode of mechanical stimulation induces an increase in the proportion of cancellous bone surfaces undertaking mineralization by a factor of approximately 10.[3] No increase in bone formation is observed in C8 of animals pinned but not loaded, nor in C6 of loaded animals.

The increase in bone formation was abolished by a single administration of indomethacin (2 mg/kg) 3 h before mechanical stimulation. The same dose of indomethacin given 3 h after loading showed no effect.[5] However, daily administration of indomethacin starting 3 h after the mechanical stimulus was also able to abolish the response. This

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suggests that there are two distinct phases of the osteogenic response that depend upon prostaglandin (PG) production: the immediate response to mechanical stimulation, which is completed within 3–6 h after loading; and a later phase.

We also found that N-monomethyl-L-arginine (L-NMMA) (30 mg/kg), a competitive inhibitor of nitric oxide synthase (NOS), suppresses the osteogenic response if administered 15 min before the episode of mechanical stimulation.[6] The suppression was largely lost when the inhibitor was administered 2 h after loading. Suppression of bone formation was prevented by co-administration of L-arginine, the normal substrate for nitric oxide production, but not by the inactive isomer, D-arginine. The effects of nitric oxide (NO) inhibition appeared specific for the mechanical response, since no changes in bone formation were induced by the NOS inhibitor in bones such as the tibia of mechanically-stimulated animals, or in non-loaded animals.

The dose of L-NMMA given was sufficient to inhibit NO production in rats for 30–60 min. Thus, in animals given L-NMMA 15 min prior to loading, NOS would be inhibited for up to 45 min after mechanical stimulation. This suggests that a form of NOS constituitively present (cNOS) is responsible for the loading response.

The induction of bone formation by a temporally well-defined stimulus in this model enables us to analyze the sequence of molecular signals associated with the osteogenic response. We analyzed this by *in-situ* hybridization and Northern analysis of RNA extracted from vertebrae. Starting approximately 9 h after loading, there was an increase in the percentage of cancellous bone surfaces showing expression, by *in-situ* hybridization, of mRNA for matrix proteins (collagen I and osteocalcin).[7] This was accompanied by a similar increase in IGF-I. Surface expression was maximal 72 h after stimulation, and returned to control levels by 120 h.

We also noted an increase in *c-fos* and IGF-I mRNA expression at earlier time points. Neither gene was detectable in osteocytes of non-loaded control bones, but both were strongly expressed within 30 min of mechanical stimulation.[8] IGF-I was detected over cortical osteocytes, particularly in the central cortex of the mid-diaphysis, and over osteocytes in cancellous bone. The percentage of osteocytes hybridizing against IGF-I increased to a maximum approximately 6 h after stimulation. *c-fos* mRNA was strongly expressed over cortical osteocytes and a proportion of cortical and trabecular bone surfaces, within 30 min, and had disappeared by 6 h. Osteocytic IGF-I, but not *c-fos,* was strongly suppressed by indomethacin administration.

The interactions between NO and PG production, and the role of *c-fos* and IGF-I expression in the induction of bone formation, remains unclear. There is much evidence that PGs can induce bone formation (see [9]). Moreover, both the induction of bone formation, and the increase in osteocytic G6PD activity after mechanical stimulation, are suppressed by indomethacin.[5,10] These observations raise the possibility that mechanically-induced bone formation is dependent upon NO production, which in turn is dependent upon PG production. Alternatively, NO might mediate bone formation through PG release, as occurs in the control of LHRH.[11] Other interactions are also possible, and the relationships between mechanical stimulation, PG and NO production, and osteocytic *c-fos* and IGF-I expression, and bone formation require further investigation.

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